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Estimation of the target stem-cell population size in chronic myeloid leukemogenesis

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Abstract Estimation of the number of hematopoietic stem cells capable of causing chronic myeloid leukemia (CML) is relevant to the development of biologically based risk models of radiation-induced CML. Through a comparison of the age structure of CML incidence data from the Surveillance, Epidemiology, and End Results (SEER) Program and the age structure of chromosomal translocations found in healthy subjects, the number of CML target stem cells is estimated for individuals above 20 years of age. The estimation involves three steps. First, CML incidence among adults is fit to an exponentially increasing function of age. Next, assuming a relatively short waiting time distribution between BCR-ABL induction and the appearance of CML, an exponential age function with rate constants fixed to the values found for CML is fitted to the translocation data. Finally, assuming that translocations are equally likely to occur between any two points in the genome, the parameter estimates found in the first two steps are used to estimate the number of target stem cells for CML. The population-averaged estimates of this number are found to be 1.86×10^8 for men and 1.21×10^8 for women; the 95% confidence intervals of these estimates are (1.34×10^8, 2.50×10^8) and (0.84×10^8, 1.83×10^8), respectively.

Key words Cancer risk modelling · Chronic myeloid leukemia (CML) incidence · CML target stem cells · genome translocation · Low-dose exposure · Radiation-induced CML

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Introduction

Estimates of cancer risks following low-dose exposures to ionizing radiation are important for public health. Since risk estimates can be formed as low-dose extrapolations of cancer risk models, the models themselves are also important. In biologically based cancer risk models, it is often assumed that there are N target stem cells capable of causing a particular cancer, and that the cancer risk per person is proportional to N [1]. The problem of risk prediction can then be decomposed into a problem of estimating N, and a problem of estimating the shape of the dose-response curve (see Eq. (11) below). The scope of this paper is restricted to the estimation of N primarily for chronic myeloid leukemia (CML); in the Discussion section we also present tentative estimates of N for acute promyelocytic leukemia (APL).

Materials and methods

Study groups

Age-specific CML incidence rates were derived from the files *lymyleuk.txt* and *reg_7395.txt* provided by the Surveillance, Epidemiology, and End Results (SEER) Program [2]. These files are composed of cancer registry data from five states (Connecticut, Hawaii, Iowa, New Mexico and Utah) and four cities (San Francisco-Oakland, Detroit, Seattle and Atlanta) for each year between 1973 and 1995. A total of 7894 cases of CML coded as 205.1 by the ICD-9 international code for diseases was extracted from the file *lymyleuk.txt*. The cases were then pooled into 5-year age groups for each sex, independent of race or SEER region. From the population file *reg_7395.txt*, the number of person-years at risk within each age group and sex was determined. The resulting data are summarized in Table1.

Chromosomal translocations were measured in 94 healthy subjects by the Lawrence Livermore National Laboratory (LLNL). Of these, 91 subjects had been described in a previous study [3], while 3 were new. From

Table 1 Summary of the chronic myeloid leukemia (CML) incidence data from the Surveillance, epidemiology, and end results program (SEER)

Age group	Men		Women	
	n	Person-years	*n*	Person-years
2.5	35	19,837,662	10	18,947,235
7.5	15	19,552,337	15	18,702,321
12.5	21	20,332,438	19	19,439,770
17.5	50	20,950,665	44	20,260,909
22.5	104	21,741,525	64	21,425,803
27.5	152	22,434,947	71	22,375,565
32.5	201	21,455,235	120	21,705,378
37.5	222	18,733,740	142	19,024,169
42.5	213	16,145,091	158	16,594,890
47.5	226	13,860,745	186	14,287,515
52.5	267	12,421,871	229	13,064,042
57.5	357	11,284,165	241	12,163,709
62.5	443	10,048,208	300	11,229,837
67.5	498	8,358,845	347	10,138,436
72.5	537	6,244,108	390	8,347,505
77.5	506	4,217,783	389	6,481,405
82.5	337	2,412,788	352	4,459,756
87.5	270	1,593,724	363	3,880,853
Total	4,454	251,625,877	3,440	262,529,098

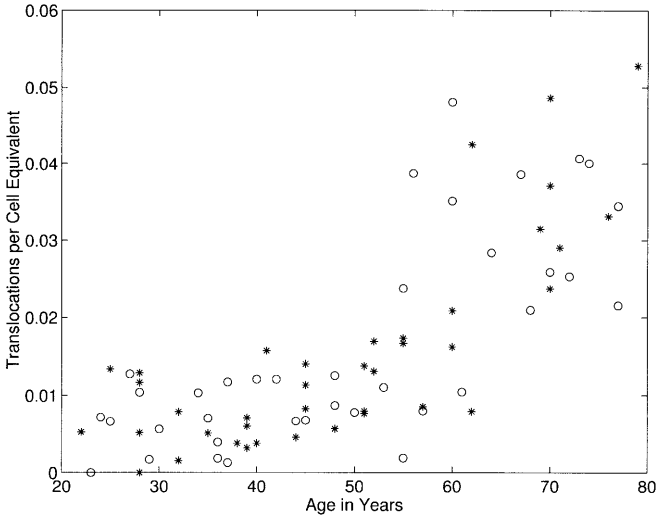

Fig. 1 Translocations per cell equivalent as a function of age greater than 20 years, men = * and women = °

the total of 94 subjects, 16 cord bloods (age 0) and one female teenager were excluded from the current study, leaving a total of 77 subjects; they were excluded because our objective is the estimation of hematopoietic stem cells in adults and "adults" are defined here as aged greater than 19 years. Translocations were measured using fluorescence in situ hybridization (FISH). Chromosomes 1, 2, and 4 were painted and scored in, on average, about 3200 metaphases per subject, yielding a G-banding equivalent of about 1100 cells per subject. Complete exchange translocations were counted as two translocations, and incomplete exchanges were scored as single translocations. A detailed questionnaire was used to

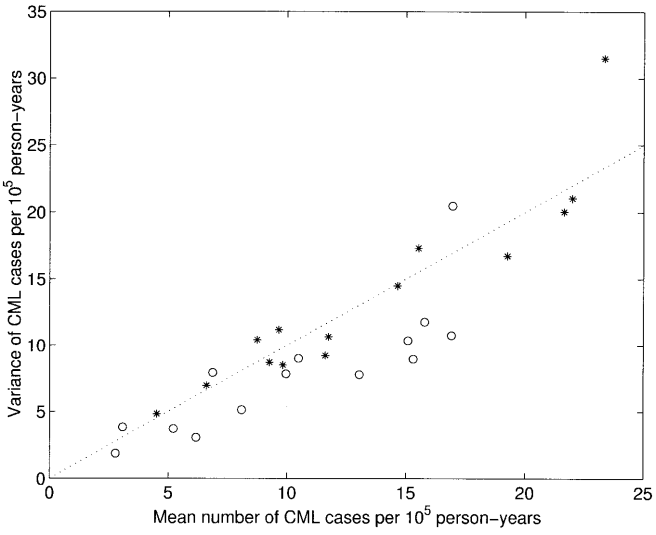

Fig. 2 The variance of CML cases as a function of their expected number, men = * and women = °. Note that the variance roughly equals the mean, i.e. that cases of CML can be assumed to be Poisson distributed; a unit slope *dotted line* is included as a reference

exclude any subjects who may have had excessive exposure, as in the treatment of previous cancers, i.e. all subjects can be considered healthy. For further details see Ramsey et al. [3]. A plot of the age structure of translocations per cell equivalent is given in Fig. 1.

Statistical methods

Parameter estimation was performed using quasi-likelihood methods for the translocation data and standard Poisson regression for the CML data. Quasi-likelihood parameter estimation, a generalization of maximum likelihood methods, does not require full specification of the distribution [4, 5]. Instead, quasi-likelihood estimation requires only that the variance-mean relationship be specified to within a constant scale factor ϕ. When the variance-mean relationship is linear with an arbitrary slope ϕ, the quasi-likelihood method reduces to Poisson regression with a free scale factor (if ϕ=1 we have a standard Poisson regression). We now examine the variance-mean relationships for the two data sets at hand.

For the CML incidence data, the variance-mean relationship appears to be linear with a scale factor ϕ=1 (Fig. 2), i.e. consistent with Poisson-distributed cases. This was determined as follows. Since no CML incidence trends were detected across the 23 calendar years between 1973 and 1995 (not shown), the variance of the number of CML cases for the *j*th age group was calculated as

$$V_j = \sum_{i=1973}^{1995} \left(O_{ji} - E_{ji}\right)^2 / (23-1) \qquad (1)$$

where O_{ji} is the observed number of CML cases in the *j*th age group and *i*th calendar year and E_{ji}, the expected number of cases, is found as

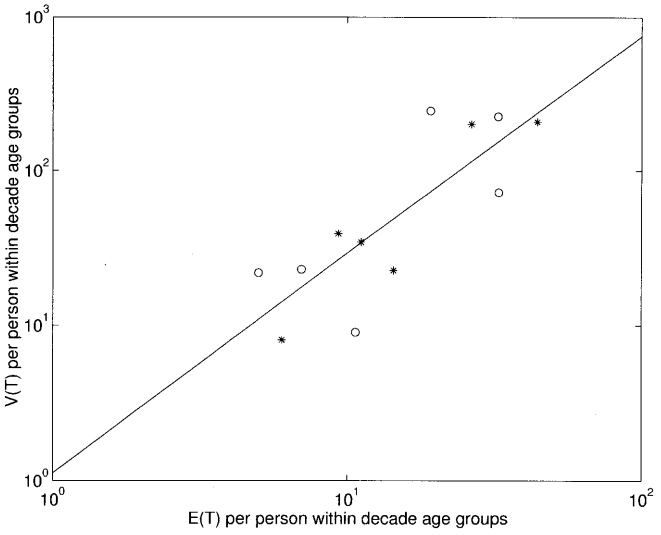

Fig. 3 The variance-mean relationship for translocations per person within decade age groups, men = * and women = °. The supralinear nature of the variance-mean relationship is readily seen as a slope >1 in this plot

$$E_{ji} = \lambda_j N_{ji} \quad \text{where} \quad \lambda_j = \frac{\sum_{i=1973}^{1995} O_{ji}}{\sum_{i=1973}^{1995} N_{ji}} \tag{2}$$

is the person-year weighted average incidence for the *j*th age group and N_{ji} is the number of person-years in the *j*th age group and *i*th calendar year. The variance V_j, plotted against the average expected number of cases $E_j=\lambda_j N_j$, where $N_j = \sum_{i=1973}^{1995} N_{ji} / 23$, gives the plot of Fig. 2.

For translocations per person, the variance-mean relationship appears to be supralinear as shown in Fig. 3. The variance calculation here was performed in a manner similar to that for cases of CML except that now individuals replace calendar years, translocations replace CML cases, and cell equivalents replace person-years at risk. The individuals were partitioned into 6 decade age groups between 20 and 80 years of age. Within each age group, an average rate of translocations per cell was calculated and multiplied by cell equivalents scored per individual to yield the expected number of translocations for each individual. The variance of translocations per person, $V(T)$, was then found for each decade as the sum of squares of observed minus expected translocations, all divided by one less than the total number of individuals for that age group. The cell-weighted average translocation rate multiplied by the average number of cells scored per individual was then taken as the mean number of translocations per person, $E(T)$. A log-log plot of $V(T)$ versus $E(T)$ is shown in Fig. 3 where each point represents one decade age group. Note that the slope in this plot corresponds to the power of the variance-mean relationship. Applying linear least squares to the transformed coordinates (with males and females pooled) yields the variance-mean relationship

$$V(T)=\phi\, E(T)^{1.4} \tag{3}$$

which we will use later in the quasi-likelihood estimation of model parameters for the translocation data. Interestingly, the same relationship with an exponent of 1.5 was found for mutations in *Salmonella* [6].

Assumptions

The target cell population size estimates derived below require several assumptions: (1) the number of CML target stem cells is constant throughout adult life; (2) the accumulation of translocations in lymphocytes is representative of the accumulation of translocations in CML target stem cells; (3) the incidence of CML is approximately rate-limited by the formation of one BCR-ABL translocation within a single CML target stem cell; and (4) translocations are equally likely to arise between any two points in the genome (this subject has recently been reviewed [7]), or, alternatively, the target DNA in BCR and ABL has, on average, a translocation probability per base-pair that approximately equals the average for the genome.

There is little evidence for or against Assumptions 1 and 2. Regarding Assumption 3, although transgenic mouse models [8, 9, 10] support the notion that BCR-ABL causes CML, BCR-ABL transcripts found in healthy adults do not [11]. One can argue that because the excess CML risk from pelvic radiation therapy vanishes in 10 to 15 years [12], all events other than BCR-ABL that are necessary for CML must be common enough to arise within this time period. In that the BCR-ABL formation rate-limits CML formation.

Results

Parameter estimates

The logarithm of age-specific CML incidence rates plotted vs age (Fig. 4) suggests the following exponential model for CML incidence rates among adults,

$$I_m = A_m e^{k_m a} \quad \text{and} \quad I_f = A_f e^{k_f a}, \tag{4}$$

where a is age in years, k_m and k_f are the aging rate constants for men and women, respectively, and A_m and A_f are amplitude parameters. Applying Poisson regression to this model using the software *AMFIT* [13] gives the parameter estimates shown in Table 2 for $a \geq 20$. The units in this table are CML cases per 10^5 person-years for A and years^{-1} for k. Note that confidence intervals for male and female estimates of A and k are non-overlapping, so males and females were not pooled. The parameter estimates for A and k are correlated with correlation coefficients of –0.963 for men and –0.964 for women, in complete agreement with the intuitive notion that as the y intercept of a fit to Fig. 4 is raised, the corresponding slope of the fit will drop, and vice-versa.

We assume that the waiting time distribution between BCR-ABL induction and CML formation is relatively

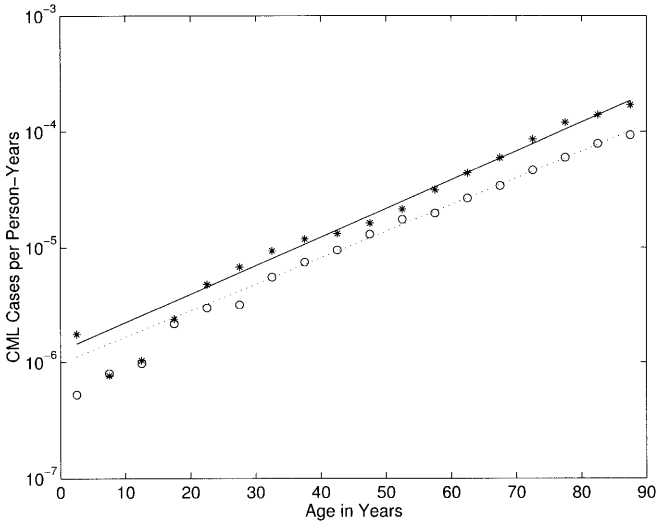

Fig. 4 CML incidence rates for all ages and exponential curve fits (Table 2) for ages ≥20 years, men = * and *solid line*, women = ° and *dotted line*. For those aged less than 20 years, the CML incidence rates lie mainly below the values predicted from the adult model fit

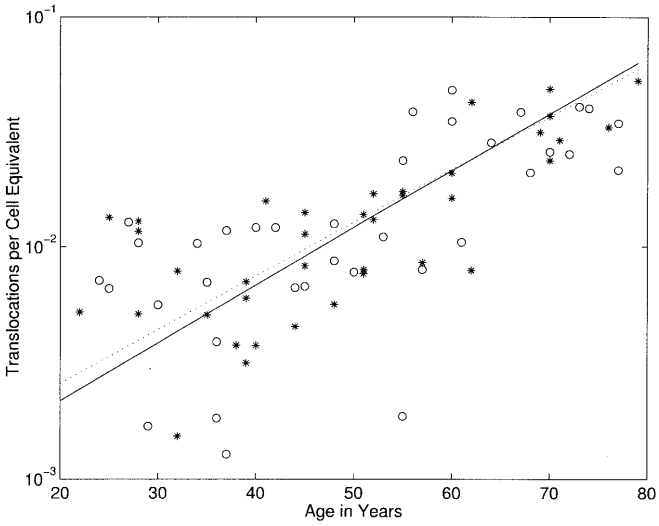

Fig. 5 The constrained quasi-likelihood fit of Eq. (5) (Table 4) to the translocation data, men = * and *solid line*, women = ° and *dotted line*

Table 2 Poisson regression parameter estimates for the CML incidence rate model of Eq. (4)

Parameter	Point estimate	95% Confidence interval
A_m	0.126	(0.113, 0.141)
k_m	0.057	(0.0554, 0.059)
A_f	0.098	(0.086, 0.111)
k_f	0.053	(0.051, 0.0548)

Table 3 Unconstrained quasi-likelihood parameter estimates for the translocation model of Eq. (5)

Parameter	Point estimate	95% Confidence interval
α_m	0.161	(0.06, 0.42)
κ_m	0.042	(0.025, 0.059)
α_f	0.169	(0.05, 0.55)
κ_f	0.041	(0.021, 0.061)

Table 4 Constrained quasi-likelihood parameter estimates for the translocation model of Eq. (5)

Parameter	Point estimate	95% Confidence interval
α_m	0.07	(0.05, 0.09)
κ_m	0.057	Fixed
α_f	0.09	(0.06, 0.12)
κ_f	0.053	Fixed

short. In this case the rate of translocation formation should have approximately the same aging rate constants as the CML incidence rate. Furthermore, since integrals of exponentials remain exponentials with the same time constants, accumulated translocations should follow

$$T_m=\alpha_m e^{\kappa_m a} \quad \text{and} \quad T_f=\alpha_f e^{\kappa_f a} \tag{5}$$

with $\kappa_m=k_m$ and $\kappa_f=k_f$. Supporting this, the unconstrained quasi-likelihood fit shown in Table 3 has 95% confidence intervals for κ_m and κ_f that include k_m and k_f. The constrained quasi-likelihood fit given in Table 4 is shown to provide a reasonable fit when plotted against the data in Fig. 5. Units for the parameter values shown in Tables 3 and 4 are translocations per 100 cell-equivalents scored for α and years^{-1} for κ and k.

Stem-cell number estimates

To estimate the CML target stem cell population sizes N_m and N_f, we assume that a translocation is equally likely to arise between any two points in the genome, that the BCR target size T_{BCR} is 5.8 kbp [14], that the ABL target size T_{ABL} is 300 kbp [14], and that the total human genome size Γ is 3200 Mb [15]. In this case the conditional probability that a translocated cell has BCR-ABL is

$$P(ba|t)=\frac{T_{BCR}T_{ABL}}{\Gamma^2}\approx 1.7\times 10^{-10}. \tag{6}$$

In words, this is the probability that one of the misrejoining DNA double-strand breaks (DSB) lands on the BCR target, T_{BCR}/Γ, times the probability that the other misrejoining DSB lands on the ABL target, T_{ABL}/Γ. Though only half of such events make BCR-ABL, the other half making ABL-BCR, an additional factor of two arises because the ordering of the two misrejoining DSBs is immaterial, so Eq. (6) holds; for a different perspective on the additional factor of two, note that if both target loci were the same size, the first misrejoining DSB would have twice the available target size as the second misrejoining DSB.

If derivatives with respect to age of Eq. (5) are multiplied by $P(ba|t)N$ and the results equated to the SEER CML incidence rates fit by Eq. (4), estimates of N_m and N_f can be found as

$$P(ba|t)N_m\alpha_m k_m e^{k_m a} = A_m e^{k_m a} \tag{7}$$

$$\Rightarrow \quad N_m = \frac{A_m}{P(ba|t)\alpha_m k_m} \tag{8}$$

and

$$N_f = \frac{A_f}{P(ba|t)\alpha_f k_f}. \tag{9}$$

Substituting the point estimates of α from Table 4 and A and k from Table 2, the CML target stem cell population sizes are estimated to be N_m=1.86×10^8 for men and N_f=1.21×10^8 for women. To obtain approximate 95% confidence intervals for these estimates, we note that upper bounds on N arise when A is at its upper bound and k is at its lower bound, while lower bounds on N arise for exactly the opposite conditions: a correlation coefficient of –0.96 between these parameters allows us to assume that when A is at its upper bound, the corresponding k will be at its lower bound, and vice-versa. Thus, by recomputing confidence limits on α using the upper and lower limits of k, and then using the bound on α that maximizes our uncertainty in N, we obtained 95% confidence intervals of (1.34×10^8, 2.50×10^8) for N_m and (0.84×10^8, 1.83×10^8) for N_f. Through Monte Carlo simulations that assume multivariate normal parameter distributions, these confidence intervals can be made even narrower (not shown). However, uncertainty in the modeling assumptions is comparatively large, so the intervals should actually be broader. For example, modeling the probability density of the time between BCR-ABL formation and CML as

$$w(t) = \frac{k_t^3 t^2}{2} e^{-k_t t}, \tag{10}$$

a fit to the CML data in Fig. 2 of [12] gave k_t=0.377 y^{-1} with a 95% confidence interval of (0.142 y^{-1}, 1.001 y^{-1}). Simulations in which the CML incidence is lagged by $w(t)$ indicate that the effect of modeling $w(t)$ would be to increase the stem cell estimates by a factor between 1.2 and 2.7. It is reasonable to expect that if the other modeling assumptions were stated in less certain forms, analogous to the use of $w(t)$, the confidence intervals for N would broaden even further.

Discussion

Estimation of N is important because of the role it plays in models of carcinogenesis. For example, a reasonable dose-response model for the excess risk of radiation-induced CML is

$$P(cml|D)=NP(ba|t)(\alpha D+\beta D^2)e^{-(\alpha_k D+\beta_k D^2)} \tag{11}$$

where $\alpha D+\beta D^2$ is the expected number of translocations per target stem cell, $\alpha_k D+\beta_k D^2$ is the expected number of lethal DSB misrejoinings per target stem cell, and D is the radiation dose in Gy. Equation (11) thus states that the probability of CML induction resulting from a dose of ionizing radiation D equals N times the probability that a target cell incurs a BCR-ABL misrejoining, multiplied by the probability that the cell has zero lethal hits. Because N is estimated in this paper using data independent of the A-bomb survivor data, it represents information which, in a Bayesian approach to parameter estimation, can potentially be brought to bear on low-dose risk estimation.

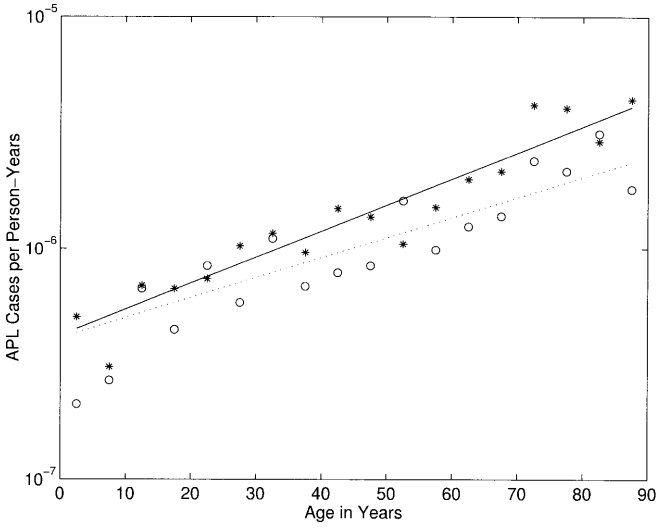

Fig. 6 Acute promyelocytic leukemia (APL) incidence rates for all ages and exponential curve fits (Table5) for ages ≥20 years, men = * and *solid line*, women = ° and *dotted line*

Our estimates of the CML target stem cell population sizes, N_m=1.86×10^8 in adult men and N_f=1.21×10^8 in adult women, are consistent with estimates in the range of 5×10^7 to 3×10^8 obtained through a comparison of the CML dose-response among A-bomb survivors and the dose-response of radiation-induced chromosomal translocations measured in vitro [Radivoyevitch et al., manuscript submitted]. They are also consistent with an estimate given by Mielcarek et al. [16] (p 230) of approximately 10^8 stem cells, but somewhat less consistent with the subsequent discussion panel's estimate of 10^7 stem cells (p 234; same paper). The characteristic features of the CML target cell population are its absolute size and the male:female ratio. Whether these features correlate with the hematopoietic stem cell populations defined by other criteria remains to be explored.

Our approach should be applicable to other leukemias mediated by single, specific translocations. To test this, 534 cases of acute promyelocytic leukemia were extracted from the SEER database to create Fig. 6. This plot is consistent with an exponentially increasing incidence for adults, just as was seen for CML in Fig. 4. However, when fitting Eq. (4) to the APL data (for support of Assumption 3, see [17, 18]), aging rate constants significantly lower than for CML arise (compare Tables 2 and 5). We have no explanation for this quantitative discrep-

Table 5 Poisson regression parameter estimates for the APL incidence rates using Eq. (5)

Parameter	Point estimate	95% Confidence interval
A_m	0.042	(0.029, 0.061)
k_m	0.026	(0.019, 0.033)
A_f	0.041	(0.028, 0.061)
k_f	0.020	(0.013, 0.028)

ancy with our model. If we ignore it on the grounds that the APL aging constants are not completely inconsistent with the translocation data, we can proceed to apply Eqs. (8) and (9) with translocation target sizes T_{PML}=7 kbp and T_{RARA}=3.5 kbp inferred from Fig. 1 of Tashiro et al. [19]. The resulting APL target cell population size estimates are then 1.8×10^9 for men and 1.6×10^9 for women, with respective 95% confidence intervals of (1.1×10^9, 3.4×10^9) and (0.85×10^9, 3.77×10^9). Since the APL target pool is likely to be more differentiated than the CML target pool, a 10-fold expansion in the target cell population size between CML and APL seems very reasonable. Furthermore, since the female:male ratio N_f/N_m rises from 0.65 in CML to 0.89 in APL, our model predicts that sexual differences in blood cell population sizes become less drastic as cells become more differentiated; in view of complete blood cell count reference ranges for male and female adults, this also seems reasonable. Though these findings appear self-consistent, they remain tentative because the differences in CML and APL aging rate constants have yet to be explained.

Note added in proof

Chromosomal translocations were not measured in subjects previously treated for cancer (see Materials and methods). Thus, to relate the translocation data to the SEER data, were should restrict CML cases to primary cancers, and we should restrict person-years to individuals with no history of cancer. These restrictions were applied (non-trivially) to the 1992–1996 SEER database (release April 1999). Using the ICD-O code 9863 to identify 1974 cases of CML, rather than the ICD-9 code 205.1 which also includes chronic myelomonocytic leukemia, male and female CML incidence rates among adults were separately fitted to the exponential age structures of Eq. (4). The results, k_m=0.04241 and k_f=0.04244 (compare with Table 2), are in exact agreement with the translocation aging rate constants of Table 3. Interestingly, the parameter correlations are such that these substantive changes in the ageing rate constants lead to only negligible perturbations in stem-cell number estimates.

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